

Diethyltoluamide (DEET) increases CD63 expression in a contact urticaria patient's basophils

To the Editor:

The use of insect repellent has increased during recent years to prevent insect stings and its feared consequences of virus and parasitic diseases transmitted by infected mosquitoes and tick bites. DEET (N, N-diethyl-3-toluamide) is the most effective and most widely-used insect repellent and it is used by approximately 30% of the population.^{1,2}

The DEET Registry is a post-marketing surveillance system which has collected voluntary reports of different and infrequent moderate to severe adverse events to this drug, including seizures and other neurological symptoms, dermal rashes and other systemic manifestations. Hives, rashes, itching, redness and swelling after exposure were detected in 85 (35%) out of 242 cases.² Hypersensitivity reactions manifested as contact urticaria and anaphylaxis, after brief contact with DEET, have also been described as case reports.³⁻⁶ In some patients with hypersensitivity to the product, skin test with DEET induced a typical immediate wheal and flare reaction.^{3,5} Furthermore, passive human sensitisation was reported positive, suggesting an IgE mediated phenomena.^{3,5} However, a skin test negative patient has also been reported.⁷

Contact urticaria has been described for a number of stimuli. Allergens from shrimp or latex react through an IgE mediated reaction, while others, like water induced urticaria or contrast media reactions, are unlikely to represent an IgE mediated response.⁸ Flow cytometry detection of basophile activation has been utilised previously for the diagnosis of allergy.⁹ Basophile activation test (BAT) takes advantage of CD63 over expression by activated basophiles. It has been described as a useful tool to explore the capacity of a substance to mediate cell activation and to suggest the possible mechanistic way of cell degranulation. Furthermore, for a number of allergens, IgE induced-basophile degranulation has been well documented. However, for pseudo-allergens, like non-steroidal anti-inflammatory drugs, basophile activation does not seem to increase CD63 or other cell membrane markers expression. So, BAT response of basophiles from a sensitive patient to DEET may contribute to our understanding of the physiology of DEET hypersensitivity reaction.

A 50-year-old woman consulted with a history of an urticarial reaction to DEET-based insect repellents. She stated that a few minutes after either spray, aerosol or lotion product application, an urticarial rash appeared on the exposed areas. She tried several different brands of repellent with the same result. Since she frequents a mosquito-infested zone she decided to consult. An open-label challenge was performed spraying a DEET containing product on her right antecubital fossae. In a few minutes, an urticarial and pruriginous rash developed which lasted for 60 min.

We evaluated the CD63 and CD11b expression on basophiles from the patient after incubation of 100 μ l of

Table 1 BAT results: Percentages of basophiles expressing CD63 or CD11b in the DEET sensitive patient and a tolerant donor.

Treatment	Basophiles CD63+		Basophiles CD11b++	
	Donor	Patient	Donor	Patient
Control	1.64	0.9	1.52	1.5
fMLP	12.30	6.51	ND	ND
DEET 1/100	0.41	13.95	0.10	9.96
DEET 1/1000	0.59	25	0.56	36
DEET 1/10000	0.58	9.51	1.02	2.17

Basophiles from peripheral blood were incubated with Buffer (Control), different dilutions of DEET or fMLP (Positive control). Cells were stained with anti-CD63 or anti-CD11b/anti-IgE/anti-CD45 and analysed by Flow Cytometry.

peripheral blood with DEET (1/100, 1/1000 or 1/10000) during 30 min at 37 °C. Cells in PBS (Phosphate Buffer Saline) and stimulated with fMLP (formyl-metionyl-leucil phenylalanine) were used, respectively, as negative and positive controls. Blood from a DEET-tolerant healthy donor was studied simultaneously. After incubation, cells were labelled with monoclonal antibodies anti-CD63 FITC (clone H5C6) or anti-CD11b FITC (clone Bear1), anti-CD45 PE-Cy5 (clone IMMU19.2) and biotinylated polyclonal anti-human IgE made in goat (Vector) followed by PE streptavidin and analysed with a FACScan flow cytometer (Becton Dickinson). **Figure 1** displays dot plots showing the isolated basophiles according to their forward (FSC) and side (SSC) scatter characteristics (R1) and anti-IgE/anti-CD45 fluorescence (R2). The percentages of activated basophiles (IgE++CD63+ or IgE++CD11b++) were recorded (**Table 1**). The drug clearly activated patient's basophiles demonstrating specific DEET hypersensitivity.

We also explored DEET interaction with T and B lymphocytes. An in vitro proliferative response to DEET was studied performing 5, 6-carboxyfluorescein diacetate succinimidyl ester-based (CFSE) proliferative assay as described.¹⁰ Cells were stimulated with 1/100, 1/1000 and 1/5000 DEET dilutions and without the drug as negative control. Stimulation with Tetanus Toxoid (TT) was used as positive control. After that, cells were aliquoted in two equal volumes and labelled with anti-CD4 PE (clone SK3)/anti-CD3 PECy5 (clone UCHT1) for T lymphocytes, and anti-CD19 PECy5 (clone HIB19) for B lymphocytes respectively. The percentages of DEET-reactive lymphocytes were recorded as those with lower CFSE fluorescence intensity as compared to non-stimulated cells, showing a homogenous bright CFSE label. TT induced 7% of proliferation compared to 1% in the negative control. Patient's PBMC proliferative responses to DEET were negative with all doses and similar to those of a DEET tolerant donor.

We conclude that DEET hypersensitivity in our patient is an IgE mediated response and that the intimate physiology of the reaction takes place inducing mast cell and basophile degranulation in a way which increases CD63 expression. T and B cell proliferative responses did not suggest direct DEET lymphocyte activation.

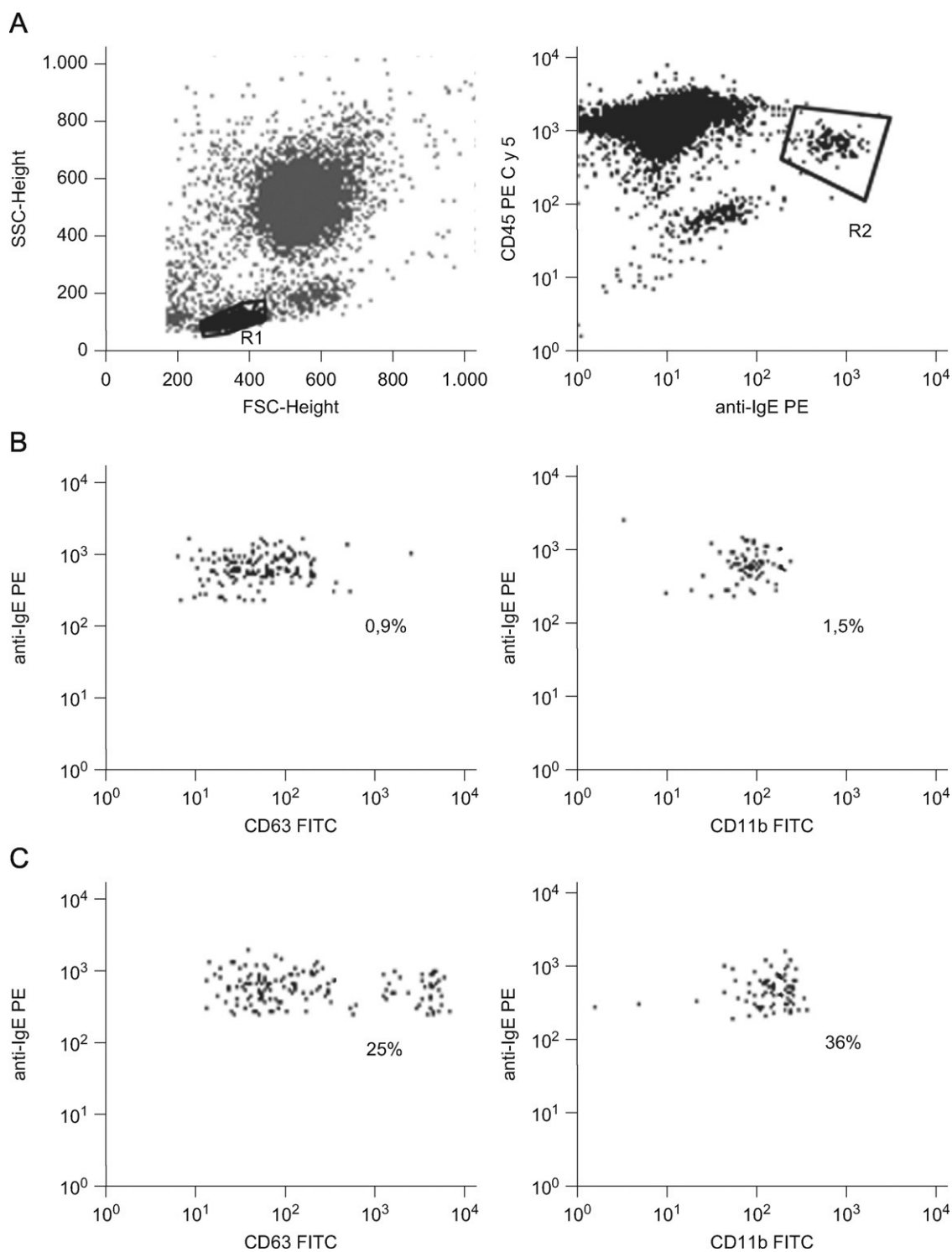


Figure 1 Flow Cytometry analysis of basophiles from the DEET sensitive patient stimulated with the drug. A: Basophiles were identified in R1 according to FSC-SSC characteristics and their staining with anti-IgE/anti-CD45 fluorescence (R2). B and C: Basophiles expressing CD63 and CD11b in the negative control (B) and after DEET stimulation (C). Percentages are indicated.

References

1. Fradin MS, Day JF. Comparative Efficacy of Insect Repellents against Mosquito Bites. *N Engl J Med.* 2002;347:13–8.
2. Osimitz TG, Murphy JV, Fell LA, Page B. Adverse events associated with the use of insect repellents containing N,N-diethyl-m-toluamide (DEET). *Regul Toxicol Pharmacol.* 2009. Sep 12. [Epub ahead of print].

3. Maibach H.J., Johnson H.L. Contact urticaria syndrome. Contact urticaria to diethyltoluamide. Arch Dermatol 1975;111:726-730.
4. Miller JD. Anaphylaxis associated with insect repellent. N Engl J Med. 1982;307(21):1341-2.
5. Von Mayenburg J, Rakoski J. Contact urticaria to diethyltoluamide. Contact Dermatitis. 1983;9(2):171.
6. Vozmediano JM, Armario J, Gonzalez-Cabrerizo A. Immunologic contact urticaria from diethyltoluamide. Int J Dermatol. 2000;39(11):876-7.
7. Wantke F., Focke H., Hemmer W., et al. Generalized urticaria induced by diethyltoluamide containing insect repellent in a child. Contact Dermatitis 1996;35:186-187.
8. Fisher A.A. Contact urticaria. In: Contact Dermatitis, 4th ed. Baltimore: Williams Wilkins, 1995;778-803.
9. Ebo D, Sainte-Laudy J, Bridts C, Mertens C, Hagendorens M, Schuerwegh A, et al. Flow-assisted allergy diagnosis: current applications and future perspectives. Allergy. 2006;61:1028-39.
10. Lyons A. Analysing cell division in vivo and in vitro using flow cytometric measurement of CFSE dye dilution. J Immunol Methods. 2000;243:147-54.

N. Galassi^a, D.S. Fernández Romero^{b,c,*}, E. Yeyati^b, A. Malbrán^{b,c}

^a *Academia Nacional de Medicina de Buenos Aires, Argentina*

^b *Unidad de Alergia, Asma e Inmunología Clínica*

^c *Servicio de Alergia e Inmunología Clínica, Hospital Británico de Buenos Aires, Argentina*

* Corresponding author.

E-mail address: dfromero@live.com.ar (D.S. Fernández Romero).

doi:10.1016/j.aller.2010.03.007

Successful venom immunotherapy to paper wasp, in IgE-venom negative patient

To the Editor:

Venom immunotherapy (VIT) is indicated only in patients with anaphylaxis and detectable venom IgE.^{1,2} However, some patients with severe anaphylaxis to insect stings, with negative skin test and RAST, need more than a set of adrenaline for protection.

A 44-year-old male roof maker was stung by a paper wasp during his work on a roof (common place of paper wasp nest) and after 30 minutes flushing of face, dizziness and difficulty in breathing occurred. A week later, another sting of an unknown insect caused no reaction at that time. It might have been a common wasp sting because it occurred in an open market place. A month later, another paper wasp sting occurred during his work and immediately he felt flushing, dizziness, difficulty in breathing and he lost consciousness within 10 min. Thanks to his profession he was able to recognise the culprit insect and in addition he brought us the polistes nest. Intradermal skin tests were negative in venom at concentration up to 1 µ/ml for all insects. RAST to all insect venoms were negative too. Serum tryptase was 9 µg/ml.

Three and six months later venom skin test and RAST remained negative. Diagnostic challenge with paper wasp sting was not carried out for ethical reasons. Although venom immunotherapy was not indicated according to recent guidelines, in this case we decided to perform it because of the severity of the reaction, the convincing history and his frequent exposure at his work place.² VIT was carried out with paper wasp venom (Pharmalgen ALK), and no reactions occurred. Paper wasp venom 100 µg as maintenance dose, every four weeks, had been administered.

A year later we challenged him with paper wasp sting in order to check the effectiveness of VIT but some concern was raised about the treatment's success because venom preparation was derived from American polistes which seems to differ from European polistes venom in the structure of antigen 5 and protease.³ Despite that the patient tolerated the challenge test with two paper wasp stings in a 15 min interval. Challenge with two common wasp stings did not cause any reaction either.

According to EAACI-position paper, patients with anaphylaxis to hymenoptera sting but undetectable IgE to venom are not recommended to receive VIT.^{1,2} Contrary to the common belief that skin test is positive in the majority of patients with a clear history of sting anaphylaxis, some studies showed negative skin test responses in up to 32% of patients with a convincing history.⁴ This low sensitivity of the skin test and RAST might be the cause of undetectable venom specific IgE in our case. A supposed sensitization in an epitope existed only in European polistes venom but not in American commercial polistes venom did not occur in our case.³ This group of IgE venom negative patients should not be ignored. In an interesting study the frequency of a future systemic reaction as it has been assessed by challenge test was the same in patients with convincing positive history, regardless of their skin test response to venom (21% in patients with positive skin test, and 22% in those with negative skin test).⁴ The repetition of skin test and RAST three and six months later does not usually solve the problem. FAST (basophilic activation tests) may offer some help.⁵ Western blot may be an alternative sensitive method but more studies are needed to verify this concept. Molecular components of polistes venom are not available.

Published practice guidelines and parameters state that patients with negative skin test responses are not candidates for immunotherapy, but no guidance is provided for managing these patients.^{1,2} Skin test and RAST with venom seem to lack the necessary sensitivity to detect low level of venom specific IgE, thus we consider that venom immunotherapy may be a challenging option in patients with undetectable venom IgE, but only when the following criteria are fulfilled: (1) frequent severe anaphylactic reactions; (2) definite recognition of the offending insect; and (3) a job-related exposure. Venom immunotherapy was proven to be successful in this case because the patient tolerated two stings challenges tests with paper wasps after VIT.

Conflict of interest

We declare that none of the aforementioned authors has conflict of interest.